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THE EFFECT OF LIGHT ON MELILOTUS ALBA.

(Sweet Clover.)

By R. W. COPPEDGE, Mulvane.

THE *Melilotus alba*, or Sweet clover, was taken up as an interesting plant to study because it seemed able, to a remarkable degree, to adapt itself to its surroundings. It is one of the earliest plants to appear in the spring, coming up, especially on a south or southeast slope, two or three weeks before any other plant. By the last of March the leaves begin to appear, and on every bright, sunny day the work of forming starch is going on in its laboratories.

By means of its tubercles it is able to extract nitrogen from the atmosphere. We find it, therefore, in great abundance in blue shale and clay soil, where few, if any, other plants can grow.

The readiness with which the leaves and growing stem respond to the light enables the plant to protect itself from heat and cold, and keep its leaves in the best position for forming starch.

Experiments have been made by the author during the months of April and May of 1899 and 1900 to find out, if possible, just how rapid these processes were.

First, as to the nutation of leaves and stems: Kellerman, in his paper read before the Kansas Academy of Science, proved that the *Helianthus annuus* did not nutate, thus disproving popular tradition. He had only worked on plants, however, after anthesis.

Schaffner proved, by a number of experiments conducted in 1896–'97, that prior to anthesis the leaves and flower-heads nutate during the day in such a way as to stand at right angles to the sun's rays. At night the leaves drop, with points turned toward the ground, occupying a sleep position.*

W. P. Wilson and Jesse M. Greenman conducted a number of experiments along the same line on the *Melilotus alba.* Their experiments were to show the regular daily movements of the plant. They found four positions which the leaves take, three normal and one abnormal. The first or normal day position may be seen in the morning or evening. The leaves are spread out, with the blades at right angles to the sun's rays.

In the second or hot-sun position the leaves stand with their tips pointed toward the sun, the blade parallel to the sun's rays.

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* Observations on the Nutation of *Helianthus annuus*, Bot. Gaz., vol. 25, pp. 395–404.

In the third or sleep position the leaves sink down, the tips are pointed toward the ground. The two lateral leaflets lie closely against the sides of the terminal leaflet, thus exposing the least possible surface to cold and transpiration.

The fourth position was brought about by covering the plant with red glass. The sleep position was thus modified some, for in this case the leaves, instead of pointing straight down to the ground, were at an angle and pointed toward the base of stem.

The author's results have agreed with Wilson's with one or two slight exceptions. I did not perform the experiment with the red glass, but I have noticed the leaves assume that position when the plants were in pots when first taken up, before the root-hairs had grown out again.

Wilson claimed that it was moisture or transpiration that caused the leaves to assume the hot-sun position. His experiment was as follows: Two potted plants were chosen; one was covered with a bell jar; the other was left standing in the open air. The light was the same in each case. The leaves on the plant in the open assumed the hot-sun position; the leaves on the one under the bell jar did not.

I performed the same experiment several times with potted plants and with plants in the field, without being able to find any difference in the position of the leaves when they were in the saturated atmosphere under the bell jar or in the open air.

A bell jar was placed over a plant in the field at 8:30 A. M. By twelve o'clock the atmosphere under the jar was saturated, as shown by the moisture which ran down the sides of the jar. On comparing the number of leaves which had assumed the hot-sun position with those of a plant standing near by under apparently the same conditions, there was no difference in the number which had turned their tips to the sun.

The same experiment was performed by covering one branch of a plant and leaving the other open to the air, but no difference could be noticed in the number of leaves which assumed the hot-sun position on the two branches.

The angles of a number of leaves were taken after they had taken the hot-sun position, and then a bell jar was placed over the plant. In a few minutes the atmosphere under the jar was saturated, but no drop from the hot-sun position occurred during one hour and thirty minutes, as shown by the angles.

A plant was covered with a bell jar at 8:30 A. M. The air was soon saturated. At eleven o'clock the leaves took up the hot-sun position. At one o'clock the jar was removed. In a few minutes the blades hung limp on their pulvini, showing that the stomata were all open. The change was so sudden that they were not able to close, as they would under normal conditions. Transpiration went on, therefore, and the leaf had soon given up too much water to stand erect.

Three plants in the open on which measurements had been taken turned their leaves each day., at about eleven o'clock, into the hot-sun position, with angles ranging from eighty-five to ninety degrees, the ground being dry. A heavy rain came on and soaked the soil, and for the next two days the leaves did not take up the hot-sun position, although the temperature was the same, twenty to twenty-three degrees centigrade, and the light strength the same.

A potted plant in the window turned its leaves each day to the light in the hot-sun position. Then the plant was supplied with plenty of water. During the next two days the leaves did not assume the hot-sun position. The temperature in each case was about the same, and the light readings for the second day showed more light than before.

From these experiments the conclusion is that the hot-sun position depends on the amount of moisture in the soil and not on the condition of the atmosphere, and that the opening and closing of the stomata depend on the relative humidity of the atmosphere.

As will be more clearly shown later in the accounts of the experiments, nutation took place in almost the entire stem in the young plant, being most noticeable in the pulvinus and decreasing towards the roots. As the plant grows older the region of nutation grows less and less. The pulvinus is so arranged as to permit the leaf to twist in any direction, so that the blade may stand at almost any angle to its petiole.

The movement originated in the leaf blade and extended from there down the stem. That the leaf was the source of the movement was proven by shading the part below the lamina, when the nutation proceeds the same as before. When the lamina above was shaded between dark papers there was no nutation. This agrees with Schaffner's experiments on *Helianthus.*

A number of experiments were made to find whether the movements of the plants and leaves depend on the light only, or whether they simply had a regular daily movement. A few experiments were sufficient to show that the plants would move in any direction to face the light, and that light alone was the cause of the normal day movements.

Schaffner says that the *Helianthus annuus* begins to nutate toward the east before the morning light could affect it. The *Melilotus alba*, however, moves only when acted upon by light. It will remain constant for days facing one direction if the light comes from that direction. It will change the position of its leaves very quickly if the direction of the incident ray is changed.

After these points were determined, an attempt was made to discover, if possible, the relation existing between the rate of movement and the strength of the light. Several methods were used in these experiments. Some healthy potted plants were placed in the laboratory window. The leaves soon all turned their faces to the light. The pot was then turned half-way round. The leaves then twisted on their pulvini, rose or fell, the stem changed its curve, so that in about thirty minutes to one hour the leaves were all again facing the light.

The easiest movement, judging from the time required, was a twist at the pulvinus. The second was where the leaf merely fell or dropped down. The one requiring the most time was where the leaf had to rise against gravity. This latter movement was accelerated when the leaf was lifted either by the wind or one's finger. This accounts for the sudden jumps which will be seen in the record of the movement of leaves taken when the wind was blowing.

Second method: A box sufficiently large not to touch the plant was taken, one end removed, and the inside lined with dark paper. This was then placed over a plant in the field, with the end left open. The light thus came to the plant from one direction only.

In some experiments the box was left over the plant during the night, facing the west, so that the leaves in the morning faced west. The box was then removed and the morning sun shone on the plant. The leaves quickly faced about to the east. Other experiments were made by placing the box over the plant at eight o'clock A. M., so that the light came from the west. The leaves at this time were all turned to the east, but they turned in a short time to the west.

The same results were secured by letting sunlight fall on the plant from the east at five o'clock in the evening, or by letting the light come from the north at noon. It was found, however, that, if the box was left over a plant for two days, those leaves which were in the dark part of the box, and were therefore not very strongly illuminated, were not able to recover from the sleep position the morning of the third day, even when exposed to the morning sunlight for two hours.

Following is a statement of some of the experiments. The angle of the leaf was measured by means of a goniometer used for determining the height of trees. The light strength was determined by means of a light machine invented by Professor Stevens, of the State University, who took a large number of the readings recorded.

Experiments made on a plant in a window:

EXPERIMENT No. 1.

Temperature, 20° C. Time, 1:30 to 2:00 P. M.
Light readings: Red, 0.372; blue, 0.328; green, 0.89.

	Leaf No. 1.	*Leaf No. 2.*
Dropped	37°	46° and 60°
Rose	5°	17°

EXPERIMENT No. 2.

Temperature, 21° C. Time, 1:00 to 3:00 P. M.
Light readings: Red, 0.605; green, 2.94; blue, 6.432.

	Leaf No. 1.	*Leaf No. 2.*	*Leaf No. 3.*
Time, 30 minutes	19° drop.	18° drop.	15° drop.
25 “	19° “	38° “	20° “
35 “	8° “	11° “	
30 “	27° “		
20 “	14° twist.	16° twist.	
20 “	20° “	30° “	
20 “	35° “	55° “	

EXPERIMENT No. 3.

Temperature, 22° C. Time, April 3, 8:25 A. M.
Light readings: Red, 0.3477; green, 1.404; blue, 3.65.

	Leaf No. 1.	*Leaf No. 2.*
Time, 60 minutes	13°	30°

Petiole of leaf twisted.

Time, 30 minntes	18°	20°

EXPERIMENT No. 4.

Temperature, 17° C. Time, April 4, 8:55 A. M.
Light readings: Red, 0.295; green, 1; blue, 7.628.

	Leaf No. 1.	*Leaf No. 2.*	*Leaf No. 3.*
Time, 35 minutes	20° drop.	20°	6°
30 “	13° “	12°	9°

Experiments made on a plant in the field (the leaves were turned to the west, because a box had been placed over the plant):

EXPERIMENT No. 1.

Temperature, 18° C. Time, April 26, 8:35 A. M.
Light readings: Red, 8.33; green, 22.75; blue, 43.73; yellow, 13.16.
Leaf rose 8°, fell 18°; stem moved 15° in 15 minutes.

EXPERIMENT NO. 2.

Temperature, 23° C. Time, May 7, 9:00 A. M.
Light readings: Red, 1.404; blue, 28.63; green, 4.31.

	Leaf No. 1.	*Leaf No. 2.*	*Leaf No. 3.*	*Leaf No. 4.*
Time, 35 minutes	15° drop.	15° drop.	20° drop.	25° twist.
25 "	14° "	13° "	15° "	20° "

EXPERIMENT NO. 3.

Temperature, 23° C. Time, May 8, 8:55 A. M.
Light readings: Red, 5.614; green, 50; blue, 10.93.

	Leaf No. 1.	*Leaf No. 2.*	*Stem rose.*	*Stem twist.*
Time, 40 minutes	12°	9°	10°	40°
20 "	5°	8°	7°	20°
5 "	4°	4°		
5 "	7°	3°		
10 "	9°			

EXPERIMENT NO. 4.

Temperature, 23° C. Time, April 23, 8:35 A. M.
Light readings: Red, 4.31; green, 65.56; blue, 43.73.

	Leaf rose.	*No. 1. Leaf fell.*	*Leaf fell.*
Time, 20 minutes	7°	30°	27°
20 "	15°	33°	30°
20 "	10°	20°	
40 "	39°	40°	
5 "	7°		
11 "	11°		
12 "	12°		
13 "	15°		

Twist of petiole, 40° in 40 minutes, and 50° in 50 minutes.
Twist of stem, 8° in 30 minutes, and 9° in 40 minutes.

Some experiments were made to determine the mutual relation of light and carbon dioxide gas in forming starch. They were as follows: Stomata were found on both sides of the leaf in the ratio of fifteen on upper to twelve on lower, under one-quarter objective and one-inch eyepiece.

Leaves of a plant which had been covered over night, and from which all the starch had gone, were treated—one with cocoa-butter on the upper side, one with cocoa-butter on the lower side, one with cocoa-butter on both sides. After several hours, the leaves were examined for starch by cross-sectioning the lamina and treating with chloral hydrate iodine and examined under the microscope. The leaf which had cocoa-butter on the under side was found to have starch grains scattered through all the cells. The one treated with cocoa-butter on the upper side had starch scattered pretty well through all the cells, excepting the long palisade cells on the upper side. Here the grains were few. The third, which had been treated on both sides, had no starch.

The cocoa-butter was applied by warming it slightly and spreading it in a thin film over the leaf, so as to exclude all carbon dioxide gas. This experiment was performed several times, with the same results.

To be sure the light was not excluded by the cocoa-butter, an experiment was made in the same way by sealing a leaf which contained no starch between two one-inch cover-glasses. The sealing was done by fastening the edges of the cover-glasses together with cocoa-butter or salve, the butter coming in contact with the leaf at the petiole only. Thus all carbon dioxide gas was excluded, but the light had free access. After a few hours, starch was found in the other leaves of the plant but none in those so treated.

On April 12 other leaves without any starch, while yet on the plant, were placed between two squares of dark paper held in place by clasps. Thus light was excluded but air was not. The squares of paper were not as large as the leaf; so part of it was exposed to the light. When examined, starch was found only in the part exposed to the light. The part of the leaf under the black paper had no starch.

On April 29, at six o'clock P. M., some leaves which were full of starch were treated as above with cocoa-butter—some dorsal only, some ventral only, some on both sides; hence, in the latter case, all light was excluded. The next morning all the starch had gone out of all the leaves excepting those treated on both sides. These were full of starch.

On May 2 branches with the leaves full of starch were broken off and placed, at six o'clock P. M., in dark drawers. After sixteen hours the starch had gone from all the cells of the leaves excepting a few of the palisades. Parts of leaves treated in the same way did not lose their starch.

Hence, when air only was present, the starch went out of the leaf, but only when light and air both were present was starch formed

A number of experimnts were performed to determine how long it required, when conditions were favorable, for the plant to form starch.

With the temperature at 20 deg. C., and light readings, red 19, yellow 20, green 20, blue 53.06, starch formed in one hour and thirty minutes. With the temperature 19 deg. C., light readings, red 1.9, yellow 2.66, green 4, blue 10.40, a cloudy day, starch formed in two hours and fifty minutes.

Later a few spring rains fell, and the new leaves which opened out were of a much lighter green than the old ones. When the temperature was 20 deg. C., and the light readings were, red 19, yellow 20, green 20, blue 56.06, it was found that it took one hour and thirty minutes to form starch in the old leaves, and two hours and thirty minutes in the young leaves.

Comparisons of the amount of light used in the two cases were as follows: Amount of light coming to the plant was, red, 19; yellow, 20; green, 20; blue, 53.06.

Amount of light which passed through dark leaf was, red, 0.1384; yellow, 0. 2647; green, 0.6; blue, none. Amount of light which passed through colorless leaf was, red, 7.826; yellow, 0.9; green, 18.5; blue, 3.

The amount of light reflected from the two leaves was the same in each case, and was as follows: Red, 0.5538; yellow, 1.125; green, 1.5; blue, 1.154.

Hence, from these figures, we get the amount of light used by each leaf in making starch as follows:

	Dark green leaf.	*Yellow new leaf.*
Red	18.3076	10.602
Yellow	18.6103	17.955
Green	17.9	......
Blue	51.906	48.906

Some experiments were performed to determine at what time in the night the starch left the plant. On the morning of April 14, it having turned cool in the night, the temperature dropped to 10 deg. C. At seven o'clock the leaves showed starch around the sieve-tubes; none in the palisade cells.

April 28 leaves taken every hour during the night were examined. Starch grew gradually less after midnight. It was nearly all gone at four o'clock; all gone at 5:15. Temperature, 20° C.

April 29 leaves picked every hour were examined. There was a gradual decrease of starch after midnight, and at five o'clock no starch was left. Temperature, average, 20° C.

On the morning of the 12th of June similar experiments were made. Although the temperature was much higher at this time, the starch remained later in the leaves, because of the greater abundance the evening before. The leaves examined in April showed scattered grains of starch, while those examined in June showed starch in thick masses crowding all the cells.

The leaves were examined every half-hour, beginning at four o'clock. At this time the cells were crowded with starch, especially around the sieve-tubes. The outer palisade cells, next to the epidermis, showed a decrease of starch. At 4 : 30 most of the starch had gone out of the palisade cells and there was a considerable reduction in all other cells. At five o'clock starch was left only in the sieve-tubes and a little in the cells bordering them. At 5 : 30 all the starch had gone from the leaf. At six o'clock starch began to appear again in the palisade cells.

Thus it will be seen that starch disappeared first from the palisade cells and reappeared there first.